



001560-373.ST25

RECEIVED

JUN 11 2002

TECH CENTER 1600/2900

SEQUENCE LISTING

DI
<110> Ohsuye, Kazuhiro
Yabuta, Masayuki
Suzuki, Yuji

<120> Process for Producing Peptides Using a Helper Peptide

<130> 001560-373

<140> US 09/402,093

<141> 1999-09-29

<150> PCT/JP99/00406

<151> 1999-01-29

<150> JP 10-32272

<151> 1998-01-30

<160> 25

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence adjacent to a site cleaved by
enterokinase

<400> 1

Asp Asp Asp Lys

1

<210> 2

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence adjacent to a site cleaved by
blood coagulation Factor Xa

<400> 2

Ile Glu Gly Arg

1

<210> 3

<211> 7

<212> PRT

<213> Artificial Sequence

DI <220>

<223> Amino acid sequence containing a site cleaved by
renin

<400> 3

Pro Phe His Leu Leu Val Tyr
1 5

<210> 4

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of helper peptide

<400> 4

Val Asp Asp Asp Lys
1 5

<210> 5

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of helper peptide

<400> 5

Gly Cys His His His His
1 5

<210> 6

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence containing a chemically
cleaved site

<400> 6

Pro Gly Gly Arg Pro Ser Arg His Lys Arg
1 5 10

<210> 7

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of helper peptide

<400> 7

His Arg His Lys Arg Ser His His His His
 1 5 10

<210> 8

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence containing a site cleaved by
 Kex2 Protease

<400> 8

Ser Asp His Lys Arg
 1 5

<210> 9

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence containing a position cleaved
 by OmpT

<400> 9

Gln Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His
 1 5 10 15
 Arg Trp Gly Arg Ser Gly Ser
 20

<210> 10

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence containing a position cleaved
 by OmpT

<400> 10

Gln Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His
 1 5 10 15
 Gly Ser Gly Ser
 20

<210> 11

<211> 69

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence coding for an amino acid
sequence containing a site cleaved by OmpT

<221> CDS

<222> (1)...(69)

<400> 11

cag	atg	cat	ggt	tat	gac	gcg	gag	ctc	cgg	ctg	tat	cgc	cgt	cat	cac	48
Gln	Met	His	Gly	Tyr	Asp	Ala	Glu	Leu	Arg	Leu	Tyr	Arg	Arg	His	His	
1				5				10					15			

cgg	tgg	ggt	cgt	tcc	gga	tcc	69
Arg	Trp	Gly	Arg	Ser	Gly	Ser	
			20				

<210> 12

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence containing a site cleaved by
OmpT

<400> 12

Gln	Met	His	Gly	Tyr	Asp	Ala	Glu	Leu	Arg	Leu	Tyr	Arg	Arg	His	His
1				5				10					15		
Arg	Trp	Gly	Arg	Ser	Gly	Ser									
			20												

<210> 13

<211> 47

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence coding for an amino acid
sequence containing a site cleaved by OmpT

<400> 13

tgg	tat	gac	g	cgg	g	ctc	g	cct	g	tat	c	g	c	g	t	c	a	c	a	c	g	g	t	t	c	c	g	47
-----	-----	-----	---	-----	---	-----	---	-----	---	-----	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	----

<210> 14

<211> 55

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence coding for an amino acid
sequence containing a site cleaved by OmpT

<400> 14

gat	ccg	ga	aac	c	g	t	g	a	t	g	a	c	g	g	c	a	t	a	a	c	c	a	t	g	c	a		55
-----	-----	----	-----	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	--	----

<210> 15

<211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer

<400> 15
 gactcagatc ttcctgaggc cgat 24

<210> 16
 <211> 36
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer

<400> 16
 aaaggtacct tccgcatgcc gcggatgtcg agaagg 36

<210> 17
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer

<400> 17
 aggccaggaa ccgtaaaaag 20

<210> 18
 <211> 29
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer

<400> 18
 aaaatgcata gcatacgtaac cgtgcatact 29

<210> 19
 <211> 627
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Nucleotide sequence coding for a fusion protein
 comprising GLP-1, helper peptide and
 beta-galactosidase protective peptide

<221> CDS
 <222> (82)...(543)

<400> 19

```

<210> 20
<211> 154
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acid sequence of a fusion protein comprising
      GLP-1, helper peptide and beta-galactosidase
      protective peptide

<400> 20
Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Lys Asp
 1             5             10            15

```

Trp Asp Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro
 20 25 30
 Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro
 35 40 45
 Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe
 50 55 60
 Pro Ala Pro Glu Ala Val Pro Ala Ser Leu Leu Glu Ser Asp Leu Pro
 65 70 75 80
 Glu Ala Asp Thr Val Val Val Pro Ser Asn Trp Gln Met His Gly Tyr
 85 90 95
 Asp Ala Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His
 100 105 110
 His Gly Ser Gly Ser Pro Ser Arg His Pro Arg His Ala Glu Gly Thr
 115 120 125
 Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu
 130 135 140
 Phe Ile Ala Trp Leu Val Lys Gly Arg Gly
 145 150

<210> 21
 <211> 187
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Amino acid sequence of a fusion protein comprising
 GLP-1, helper peptide and beta-galactosidase
 protective peptide

<400> 21
 Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Lys Asp
 1 5 10 15
 Trp Asp Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro
 20 25 30
 Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro
 35 40 45
 Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe
 50 55 60
 Pro Ala Pro Glu Ala Val Pro Ala Ser Leu Leu Glu Ser Asp Leu Pro
 65 70 75 80
 Glu Ala Asp Thr Val Val Val Pro Ser Asn Trp Gln Met His Gly Tyr
 85 90 95
 Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro
 100 105 110
 Pro Phe Val Pro Thr Glu Pro His His His His His Gly Gly Arg Gln
 115 120 125
 Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His Arg
 130 135 140
 Trp Gly Arg Ser Gly Ser Pro Ser Arg His Lys Arg His Ala Glu Gly
 145 150 155 160
 Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys
 165 170 175
 Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly
 180 185

<210> 22
 <211> 184
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Amino acid sequence of a fusion protein comprising
 GLP-1, helper peptide and beta-galactosidase
 protective peptide

<400> 22

DI
 Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Lys Asp
 1 5 10 15
 Trp Asp Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro
 20 25 30
 Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro
 35 40 45
 Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe
 50 55 60
 Pro Ala Pro Glu Ala Val Pro Ala Ser Leu Leu Glu Ser Asp Leu Pro
 65 70 75 80
 Glu Ala Asp Thr Val Val Val Pro Ser Asn Trp Gln Met His Gly Tyr
 85 90 95
 Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro
 100 105 110
 Pro Phe Val Pro Thr Glu Pro His His His His His Gly Gly Arg Gln
 115 120 125
 Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His Gly
 130 135 140
 Ser Gly Ser Pro Ser Arg His Lys Arg His Ala Glu Gly Thr Phe Thr
 145 150 155 160
 Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile
 165 170 175
 Ala Trp Leu Val Lys Gly Arg Gly
 180

<210> 23
 <211> 184
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Amino acid sequence of a fusion protein comprising
 GLP-1, helper peptide and beta-galactosidase
 protective peptide

<400> 23

Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Lys Asp
 1 5 10 15
 Trp Asp Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro
 20 25 30
 Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro
 35 40 45
 Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe
 50 55 60
 Pro Ala Pro Glu Ala Val Pro Ala Ser Leu Leu Glu Ser Asp Leu Pro


```

65      70      75      80
Glu Ala Asp Thr Val Val Val Pro Ser Asn Trp Gln Met His Gly Tyr
      85      90      95
Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro
      100      105      110
Pro Phe Val Pro Thr Glu Pro His His His His His Gly Gly Arg Gln
      115      120      125
Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His Gly
      130      135      140
Ser Gly Ser Pro Ser Arg His Pro Arg His Ala Glu Gly Thr Phe Thr
145      150      155      160
Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile
      165      170      175
Ala Trp Leu Val Lys Gly Arg Gly
      180

```

DI

```

<210> 24
<211> 5
<212> PRT
<213> Artificial Sequence

```

```

<220>
<223> Amino acid sequence containing a site cleaved by
      Kex2 Protease

```

```

<400> 24
Ser Cys His Lys Arg
 1             5

```

```

<210> 25
<211> 6
<212> PRT
<213> Artificial Sequence

```

```

<220>
<223> Amino acid sequence containing a site cleaved by
      Kex2 Protease

```

```

<221> PEPTIDE
<222> 6
<223> Xaa = Gly

```

```

<400> 25
Arg His His Gly Pro Xaa
 1             5

```